Gene Therapy:

I have always been fascinated by gene therapy, the idea that one can place a healthy gene (DNA sequence) into a vector, be it virus or plasmid (a circular piece of DNA, like a mini-chromosome found in bacteria) then into a cell, or directly into a human to repair or replace a mutant gene. The ultimate aim is to "cure" specific genetic diseases by replacing the defective gene with a normal one. In practice two different approaches have been tried, in the first usually lymphocytes (a type of white blood cell) were harvested from a patient with a genetic defect, grown in culture, a normal gene added in the form of DNA to the cells to repair the affected function (this is known as transformation or transfection) and after a few rounds of cell replication, the transformed cells were transplanted into the individual from which they were originally harvested. This is known as in vitro gene therapy. This technique has been performed successfully in the case of children with adenosine deaminase (ADA-SCID) deficiency, a severe condition of the immune system. Until recently this experimental approach was not approved by the US FDA, but performed in Europe. The results have been mixed, with a high incidence of leukemia occurring in many of the patients.

In the early 1990's a second approach, known as in vivo gene therapy was developed. A specific gene is inserted by recombination and integration into a virus, for example the normal gene defective in cystic fibrosis patients, and this recombinant virus is injected directly into patients with the disease. Adenovirus -5 used in these experiments, is an innocuous virus, occasionally associated with symptoms of the common cold. This approach has been only partially successful, in part because of variable low expression of the inserted gene, and the inability to target the gene to the required organ or site in the body. The lung is a difficult organ to treat because of size and the stickiness of mucous as a result of the disease. Although theoretically simple to perform such treatments, gene therapy has had a large number of failures. The occurrence of accidental deaths, due to over-zealous treatment and/or over- zealous researchers halted research for a number of years, and led to skepticism and lack of funding for some time.

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The reason for isolating adenosine phosphoribosyl transferase (APRT) deficient mutants of mouse L-cells in my lab, was to use them as targets for gene therapy. We also cloned the aprt gene. I decided to use adenovirus-5 as a vector, previously utilized in other studies.` To test feasibility, we infected (or transfected with DNA) aprt mutant mouse L-cells with an adenovirus containing the Chinese hamster aprt gene DNA, inserted into a non-essential region of the virus (known as the E3 region) not required for viral replication. This virus was constructed by Vincent Konan, a Ph.D. student from the Ivory Coast while the vector was developed by Frank Graham of McMaster's University. Frank also developed the technique of calcium transformation, a technique in which DNA bound to calcium was taken up by cells in culture and expressed for a long time, forming what were called stable transformants (1). He also developed the techniques for cloning foreign genes into adenovirus. We used mouse L-cells since the virus does not replicate in mouse cells and thus does not destroy them, although the transgene is expressed, implying that the virus persists in the cell over a long period. These experiments were very successful, and this work formed Vincent's MA thesis. Thus we could use adenovirus to transform APRT negative mutant cells to APRT positive cells. Evidence indicated that the APRT gene was into the mouse genome (DNA) at random locations. This was the result of a double recombinant event since in those clones examined viral DNA was lost.

This project was then joined by Qing Wang, a Ph.D. student from China (Nanking). Qing was a very serious young woman. Unusual for Chinese students, she was a practicing Catholic, married and had a young child. I never met her husband, who worked as a watch repair man. They were apparently childhood sweet- hearts. The marriage did not last long. She divorced him before completing her Ph.D. and when she left Bloomington she had the custody of her son who moved with her to California.

Qing used a variety of constructs of the CHO aprt gene, and transfected known CHO mutants with the recombinant adenovirus. Such mutants after transfection had normal levels of APRT. In order to get high levels of recombinants the APRT promoter region appeared to be required. The transductants, as in the case of the L-cells described above appeared to be the result of double cross-overs, since little viral material remained. This was targeted recombination and not a random event. This was an important observation and we should have spent more time investigating the mechanism of recombination. The

fact that the aprt promoter was required for this event was also interesting since it appeared to be stronger than the viral promoter. (2)

On completion of her Ph.D. Qing took a position with Cell Genesis, a new biotech company in Foster City, South San Francisco. Its original aim was to produce vectors for gene therapy, and it later turned attention to the development of cancer vaccines (stimulation of the immune system) using viral vectors. Qing is listed as first inventor on a patent developing new adenovirus constructs. I visited Cell Genesys in the very early days of the company and gave a talk on APRT as model for gene therapy. As in the case of many biotech companies, none of the products passed phase 1 or phase 2 trials, and Cell Genesys eventually merged with another drug company after a financial collapse. Qing herself has since worked for a number of other biotech companies, married a Jewish engineer and resides in Palo Alto.

We then decided to use the consensus interferon gene, supplied by Dr. Larry Blatt of Amgen in gene therapy experiments. The idea was to clone the gene into a virus vector, deliver it directly into tumors, check for interferon activity, and examine whether it inhibited tumor growth. We first cloned the gene into a small virus, considered useful as a vector, adeno-associated virus (AAV). This is a defective virus and requires adenovirus for growth. Thus the recombinant virus was grown in the presence of adenovirus-5, and the AAV recombinants harvested and separated from adenovirus. The consensus IFN under a zinc promoter, was expressed in all the cells infected with AAV in the presence of zinc. The cloning efficiency of the tumor cell lines was greatly reduced by the interferon gene. When the transformed tumor cells were injected into nude mice, tumor growth did not occur in contrast to untransformed tumors. Likewise when established tumors were treated with other cells producing the consensus interferon, tumor growth was inhibited. Whether the tumor regression was due to the consensus interferon or to activation of other components of the immune system is unclear (3, 4).

In collaboration with Larry Blatt we constructed a number of viral vectors containing the consensus IFN gene. These were placed under different promoter sequences, and transfected into the leukemic cell line K562. Such constructs reversed the tumorgenic phenotype of the leukemic cells.

Much of this work was done by a post-doc, Yipping Geng. Yipping had the equivalent of a Chinese MD, but could not practice medicine. She worked very hard, and on leaving my lab she completed her M.D. and is now a practicing physician in the New York City area. At this particular point in time I had a very large group of students/post-docs in the lab, and the basic language was Chinese. Apart from Yipping there was Jian Zhang, another post-doctoral fellow (or visiting scholar), very gifted with his hands, Chen Ju Hu, already mentioned elsewhere and Yanling Huang my lab technician. The next stage in " adventures" in gene therapy was using the consensus interferon gene in the adenovirus vectors. Here our aim was loftier, to inject the interferon gene into nude mice with human breast cancer and look for an interferon effect on the tumor growth and size.

We injected adenovirus containing the consensus interferon gene into the tumors at different times. The end point of these experiments was tumor regression. These experiments were quite successful. Tumors regressed completely when the site was injected with adenovirus/interferon. However the tumors also regressed when injected just with the adenovirus control, but not when given saline. Interferon injection directly also had some effect on the tumors but less than the combination. Thus we appeared to looking at a viral-therapy effect as well as an effect of the interferon. (5)

This combination was never tried on humans, perhaps because of a disaster that occurred around this time at the University of Pennsylvania in the treatment of a young man with adenovirus carrying a gene constructed to reverse ornithine transcarbamylase deficiency. Two investigators, Jim Wilson and Bill Kelly treated a young man, Jesse Gelsinger, age 18, for ornithine transcarbamylase deficiency using an adenovirus construct. Gelsinger suffered from OTD an X-linked genetic disease of the liver, the symptoms of which include an inability to metabolize ammonia - a byproduct of protein breakdown. The disease is usually fatal at birth, but Gelsinger had not inherited the disease; in his case it was apparently the result of a spontaneous genetic mutation after conception and as such was not as severe - some of his cells were normal which enabled him to survive on a restricted diet and special medications. It appears that an overdose of adenovirus was used resulting in an especially severe immune response. Unfortunately the researchers made some very serious errors or judgment. They did not report adverse effects in other

patients, nor the fact that monkeys had died after a similar dose of adenovirus. As a result of this accident NIH froze all gene therapy trials for a number of years. A few years later another setback occurred using mouse leukemia virus as a vector in children suffering from combined immune-deficiency. Although for the first few years the treatment seemed to be effective, eventually four of the children developed leukemia, which resulted from insertion (recombination) of the virus into a location on the chromosome near an oncogene, thus activating this gene. This was a reflection of the randomness of the integration process. Thus gene therapy has had a very checkered history and progress was delayed as a result.

Thus, although our results were positive, tumor did regress following treatment, it was the predominantly the result of viral oncolysis and not the expression of the interferon. By this time Amgen was no longer interested in the consensus interferon, they had sold it to a Japanese company, and it was near impossible to get funding for a continuation of gene therapy, so that this project basically died as a result of a low funding priority. As stated elsewhere, I should not have given up but resubmitted the grant proposal with novel ideas. I learned only the other evening that adenovirus containing the interferon-alpha gene is being used or is in clinical trial for the treatment of bladder cancer.

Certainly during the last few years Chinese scientists and a Chinese biotech company have been using viral vectors to treat various types of cancers, in particular glioblastoma in the brain. Amgen has developed a herpes virus that destroys melanoma cells, and an AAV has been modified for the treatment of a specific eye disease. There is no doubt that this is the way of the future for specific rare genetic diseases, and even for some type of cancers.

This was a period when my lab was very busy, and a very happy place to work. I had an excellent group of students, and they interacted with each other very well, and perhaps at times were a little too zealous in trying to please me.

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